American Journal of Medical Genetics 68:409–411 (1997)

Paracentric Inversion Involving the Long Arm of Chromosome 9 Resulting in Deletion of *abl* Gene

Svetlana M. Kleyman, Aruna J. Parekh, Abraham R. Rodriguez, Robert A. Conte, and Ram S. Verma*

Division of Genetics and Department of Pediatrics, The Long Island College Hospital–SUNY Health Science Center at Brooklyn, Brooklyn, New York

We report on a new chromosomal finding in a newborn male with hypertelorism, apparently low-set malformed ears with patent canal, micrognathia with narrow high-arched palate, bilateral webbing of neck with low posterior hairline, widely spaced nipples, and complex heart anomalies. Initially, what appeared to be a simple paracentric inversion of the long arm of chromosome 9, that is, 46,XY, inv(9)(q31q34) by routine GTG-banding technique was later determined to be a paracentric inversion with deletion of the band 9q34.1 by FISH technique using an *abl* unique sequence DNA probe. Thus the cytogenetic diagnosis was modified to 46,XY,der(9) inv(9)(q31q34.1)del(q34.1). Nevertheless, the presence of telomeric repeat sequences in the inverted chromosome 9 suggests that either healing has occurred by adding $[TTAGGG]_n$ sequences to the non-telomeric end (q31) by the enzyme telomerase or telomeric sequences were not affected during this inversion process. This abnormality is a rare occurrence and has never been reported before either because of a high rate of lethality or it has been undetected by routine cytogenetic techniques. The other abnormal cases with apparent paracentric inversions could also have a complex nature with congenital anomalies associated with loss of "few" DNA sequences as exemplified here. Am. J. Med. Genet. 68: 409–411, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: chromosome 9; paracentric inversion; del(9)(q34.1); *abl* gene; FISH technique

INTRODUCTION

The incidence of paracentric inversions in the general population is 1/2,000 to 1/3,500 [Hook et al., 1984]. Generally, the regions involved in paracentric inversions do not pair during meiosis, thus, crossingover does not occur, producing no phenotypic consequences [Daniel, 1988]. Nevertheless, there are many reports that suggest various degrees of malformation with such inversions [Madan, 1995]. Obviously, the potential loss of genetic material in these previous cases was so minimal that it would have escaped detection by routine cytogenetic techniques. We report such a case where GTG banding failed to detect a microdeletion in chromosome 9. Employing the FISH technique, a deletion in chromosome 9 band q34.1 was documented by an *abl* unique sequence DNA probe. The present communication raises a serious question concerning previous reports involving potential deletions, as a result of paracentric inversions, which may have gone undetected.

MATERIALS AND METHODS

The cytogenetic analyses were performed on the peripheral blood lymphocytes of the index case and both parents. Culturing and GTG-banding procedures were according to Verma and Babu [1995]. FISH technique was employed as suggested by the manufacturer (Oncor, Gaithersburg, MD). A cocktail probe containing an *abl* oncogene unique sequence DNA probe (biotin), which is between 60 and 80 kb, and a chromosome 9-specific classical satellite DNA probe (D9Z1, biotin) were hybridized to fixed metaphase chromosomes of the propositus and his parents. To ensure a high degree of certainty, the signals were amplified using an anti-avidin antibody and a second round of fluorescein-labeled avidin was added. The FISH procedure was repeated twice more in the index case, and 100 cells were analyzed in each trial. In addition, exposure time was drastically increased up to 120 seconds using a 100-W mercury short arc lamp on a Zeiss Axiophot microscope with a FITC filter. A whole chromosome paint (WCP) probe for chromosome 9, and the human all telomere probe (biotin) with the beta satellite DNA probe for chromosome 9 (D9Z5, biotin, Oncor), were also employed. A DAPI filter was used in the WCP technique.

*Correspondence to: Dr. Ram S. Verma, Division of Genetics, The Long Island College Hospital, Hicks Street at Atlantic Avenue, Brooklyn, NY 11201.

Received 1 December 1995; Accepted 5 April 1996

CLINICAL REPORT

The index case weighed 3695 g (75th centile) at term and was delivered normally to a 28-year-old mother (G6, P3023), by normal spontaneous vaginal delivery with Apgar scores of 9/9 at 1 and 5 minutes, respectively. Physical findings at birth included: hypertelorism, apparently low-set abnormal ears with patent canals, micrognathia with narrow high arched palate, and bilateral webbing of neck with low posterior hairline. His length was 53 cm (60th centile), head circumference 34 cm (50th centile), chest was symmetrical with widely spaced nipples. The infant had a complex cardiac anomaly, including a long systolic heart murmur radiating to both axillary regions. An echocardiogram showed situs solitus with normal venous return, large ASD with left to right shunt, virtual mitral atresia with small left ventricle, large ventricular septal defects, D-transposition of the great vessels—both arising from the anterior right ventricle—and subpulmonic stenosis with antegrade flow to pulmonary arteries. The aortic arch was normal.

RESULTS AND DISCUSSION

Cytogenetic findings with GTG banding demonstrated an abnormal 46,XY, inv(9)(q31q34). The initial impression was that breakpoints were at bands q31 and q34, and the entire segment was inverted (Fig. 1). Based on this scenario, it was presumed that there was no loss of genetic material. Routine and molecular cytogenetic techniques showed that both parents were apparently normal.

The patient has multiple congenital abnormalities, which prompted us to employ the fluorescence in-situ hybridization (FISH) technique. First, metaphases were hybridized by WCP probe specific for chromosome 9. The nature of the abnormality was not determined by WCP probe. The *abl* unique sequence DNA probe was used to investigate the presence of band 9q34.1. The lack of signals on the inverted chromosome 9 after multiple trials was an apparent indication that a microdeletion had occurred in band 9q34.1 (Fig. 2). In order for a positive locus signal of a unique sequence probe to be detected at the microscopic level it must be at least 1 kb (Oncor, personal communication). Furthermore, we hybridized the metaphases with a human telomeric DNA probe and observed the presence of telomeric sequences on the inverted chromosome 9, suggesting that the deletion was interstitial. It is possible that only a few DNA sequences of the *abl* gene were truncated due to the inversion resulting in a negative signal when chromosome 9 was hybridized with the *abl* probe, but telomeres were retained. Alternatively, the broken chromosome may have been healed by the addition of the telomeric repeat sequences $[TTAGGG]_n$ to nontelomeric DNA by the enzyme telomerase [Day et al., 1993; Meltzer et al., 1993; Bertoni et al., 1994; Flint et al., 1994; Herz et al., 1995; Kipling, 1995]. Nevertheless, the cytogenetic diagnosis was changed to: 46,XY,der(9)inv(9)(q31q34.1)del(9) (q34.1).

From a clinical viewpoint, the phenotypic abnormalities associated in this case may be coincidence or due to a microdeletion within band 9q34.1 caused by an in-

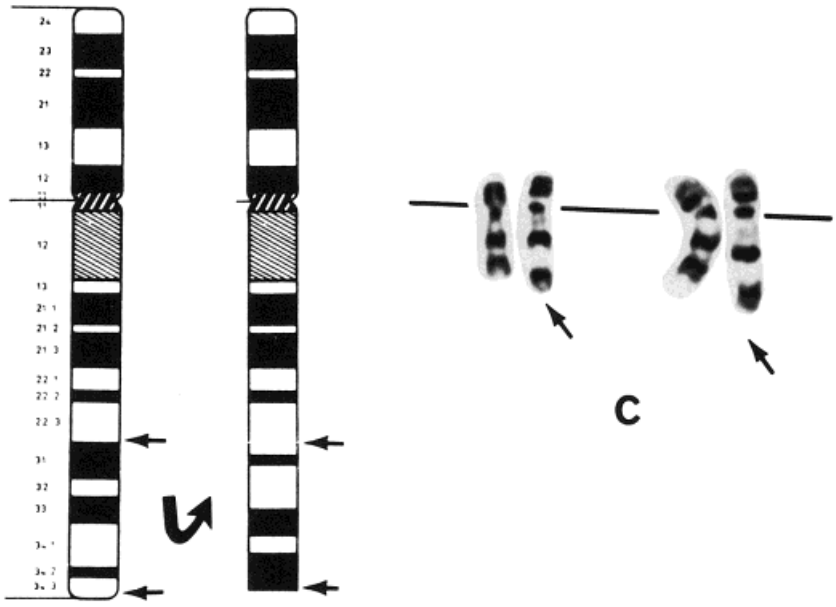

Fig. 1. **A:** Diagrammatic representation of normal chromosome 9. **B:** The inverted chromosome 9 showing deletion of band 9q34.1 is shown and breakpoints are marked with arrows. **C:** The GTG-banded chromosome 9s are shown from two cells, abnormal chromosomes 9s are shown by arrows.

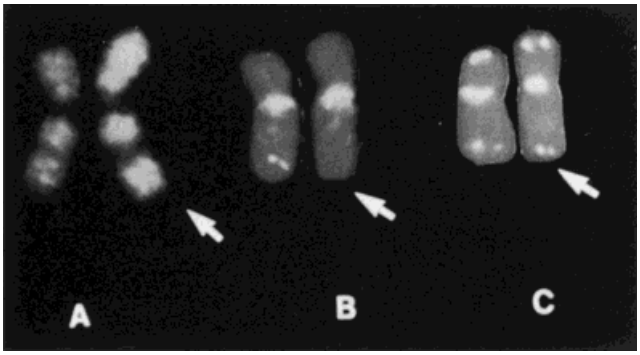

Fig. 2. Chromosome 9 stained by FISH technique. **A:** Chromosome 9 hybridized with WCP-probe. **B:** Chromosome 9 hybridized by *abl* probe with a classical satellite DNA probe for D9Z1 (note the deletion of *abl* gene). **C:** Chromosome 9 hybridized by human telomere probe with the beta satellite probe. The presence of telomeres on both chromosome 9s were noted. Abnormal chromosome 9s are marked by arrows.

version. This case is a rare abnormality and has never been reported before. The deletion of band 9q34.1 may have a high rate of lethality (as does Ullrich-Turner syndrome), and this could explain its rare occurrence. Alternatively, routine cytogenetic methods may have failed to detect it. In previous cases where a ring chromosome 9 was involved and band 9q34 was presumed to be deleted, the major manifestations included mental retardation, microcephaly, trigonocephaly, exophthalmos, anteverted nostrils, abnormal auricles, short neck, hypospadias, cryptorchidism, short stature, cardiopathy, and heart malformation [reviewed in Manovrier-Hanu et al., 1988]. Nevertheless, it must be emphasized that there may also be a partial deletion involving the terminal bands of the short and long arms during ring formation [Kasa and Kasai, 1988]. Furthermore, there are a number of reports where larger segments of 9q are deleted, ranging from q22.1 to qter having variable clinical manifestations [Turleau et al., 1978; Ying et al., 1982; Kargas et al., 1987; Zuffardi et al., 1989]. A case by Turleau and his associates [1978] with minimal interstitial deletion involving bands q32q34 had brachycephaly, a deep nasal bridge, a short nose, and hypotonia [Kargas et al., 1987, see Table I]. Consequently, comparison of our case to those having ring chromosome 9 or those involving larger deletions may be irrelevant. Nevertheless, it is tempting to speculate that in other cases of paracentric inversion the clinical abnormalities may be caused by disruption of a gene at one of the breakpoints, rather than by deletion. Molecular genetics is at the threshold of dissecting single chromosome bands and the detection of a microdeletion is a clear indication that previous cases with multiple clinical manifestations may not be characterized as a simple paracentric inversion, but may also be associated with cryptic deletions.

ACKNOWLEDGMENTS

We are grateful to Robert Robinson for preparing the photographs and to Mark A. R. Sealy for typing the manuscript.

REFERENCES

Bertoni L, Attoloini C, Tessera L, Mucciolo E, Giulotto E (1994): Telomeric and non-telomeric $(TTAGGG)_n$ sequences in gene amplification and chromosome stability. Genomics 34:53–62.

Daniel A (ed): (1988): "The Cytogenetics of Mammalian Autosomal Rearrangement." New York: Wiley-Liss.

Day JP, Marder BA, Morgan WF (1993): Telomeres and their possible role in chromosome stabilization. Environ Mol Mutagen 22:245–249.

Flint J, Craddock CF, Villegas A, Bentley DP, Williams HJ, Galanello R, Cao A, Wood WG, Ayyub H, Higgs DR (1994): Healing of broken human chromosomes by the addition of telomeric repeats. Am J Hum Genet 55:505–512.

Herz JM, Tommerup N, Sorensen FB, Henriques UV, Nielsen A, Therkelsen AJ (1995): Partial deletion 11q: Report of a case with a large terminal deletion 11q21-qter without loss of telomeric sequences, and review of the literature. Clin Genet 47:231–235.

Hook EB, Schreinemachers DM, Willey AM, Cross PK (1984): Inherited structural cytogenetic abnormalities detected incidentally in fetuses diagnosed prenatally: Frequency, parental-age associations, sex-ratio trends and comparisons with rates of mutants. Am J Hum Genet 36:422–433.

Kargas SA, Kargas GA, Chandra S, Wu S, Meisner LF (1987): Brief clinical report: Newborn infant With del(9)(pter→q32:) and multiple congenital anomalies including arrhinencephaly, cardiac malformations, and rudimentary ears. Am J Med Genet 3:161–166.

Kasa N, Kasai R (1988): A patient with ring 9 chromosome 46,XY,r(9)(q24.1q34.3). Acta Paediatr Jpn 30:710–713.

Kipling D (1995): "The Telomere." New York: Oxford University Press.

Madan K (1995): Paracentric inversions: a review. Hum Genet 96:503–515.

Manovrier-Hanu S, Turck D, Gottrand F, Savary JB, Loeuille GA, Deminatti MM, Farriaux JP (1988): Le chromosome 9 en anneau. Ann Genet 31:250–253.

Meltzer PS, Guan X-Y, Trent JM (1993): Telomere capture stabilizes chromosome breakage. Nature Genet 4:252–255.

Turleau C, de Grouchy J, Chabrolle JP (1978): Délétions intercalaires de 9q. Ann Génét 21:234–236.

Verma RS, Babu A (1995): "Human Chromosomes: Principles and Techniques." New York: McGraw-Hill.

Ying KL, Curry CJR, Rajani KB, Kassel SH, Sparkes RS (1982): De novo interstitial deletion in the long arm of chromosome 9: A new chromosome syndrome. J Med Genet 19:68–70.

Zuffardi O, Caiulo A, Marascio P, Tupler R, Bianchi E, Amisano P, Beluffi G, Moratti R, Liguri G (1989): Regional assignment of the loci for adenylate kinase to 9q32 and for α_1-acid glycoprotein to 9q31-q32. Hum Genet 82:17–19.